Similarly, claims 120, 133 and 136 have been amended to recite the duplexes of claims 75 or 76, and new claims 139-141 have been added to recite the oligonucleotide of claim 77. In light of these amendments, Applicants urge that this rejection be withdrawn.

B. Claims 100, 103, 107, 127-132 and 136-138 stand rejected over the recitation "5' end or vicinity thereof." This is a reiteration of a previous rejection, which was traversed in the previous Response. Applicants maintain that one of skill in the art would be able to determine, without undue experimentation, a region of an oligonucleotide that constitutes the vicinity of the 5' end, such that placement of a label within such region would not prevent chain extension of the labeled oligonucleotide by a polymerase.

Nonetheless, without conceding the correctness of this rejection, but simply to advance prosecution, claims 100, 103, 107, and 136-138 have been amended to remove the reference to vicinity of the 5' end. Since claims 127-129 depend, in part, from claim 107; and claims 130-132 depend, in part, from claim 103; the amendments to claims 103 and 107 remove the bases for rejection of claims 127-132. Accordingly, this rejection can be properly withdrawn.

C. Claims 120, 133 and 136 stand rejected over the recitation "duplex of claim 77," as claim 77 recites an oligonucleotide. In response, claims 120, 133 and 136 have been amended to recite the duplex of claim 75 or 76; and new claims 139-141 have been added to recite the oligonucleotide of claim 77. In addition, claims 118 and 119 have been amended to recite the oligonucleotide of claim 77. Accordingly, this rejection can be properly withdrawn.

D. Claims 121-123, 134 and 137 stand rejected over the recitation "the set of primers of claims 81 to 83," as lacking proper antecedent basis. In response, Applicants note initially that claims 121-123 recite a "set of primers"; while claims 134 and 137 recite a "set of duplexes". To more particularly point out and distinctly claim the invention, claims 121-123 have been amended to recite the set of duplexes of either of claims 81 or 82; and new claims 142-144 have been added to recite the set of oligonucleotides of claim 83. In addition, claims

134 and 137 have been amended to recite the set of duplexes of either of claims 81 or 82; while new claims 145 and 146 have been added to recite the set of oligonucleotides of claim 83. Accordingly, Applicants urge that this rejection be withdrawn.

E. Claims 76 and 98 have been amended to more particularly point out and distinctly claim the invention.

Concerning 35 U.S.C. § 103(a)

A. Claims 75-77, 81-83, 88, 98, 100-101, 103-105, 107, 109-111, 118-132 and 136-138 stand rejected under this section as being allegedly obvious over Qu or, in the alternative, over Hindley, each in view of Langer, and each further in view of Leary. Qu and Hindley are stated in the Office Action to disclose radioactively-labeled oligonucleotide primers and their extension products. Langer is stated to disclose biotinylated nucleotides and probes made with biotinylated nucleotides, and is alleged to teach detection of a biotinylated oligonucleotide using avidin coupled to a fluorescent dye. Leary is stated to disclose a method of making biotinylated probes using nick translation. It is concluded that it would not only have been possible, but obvious to make a biotinylated nucleic acid probe by nick translation, which could allegedly have been detected using fluorescently labeled avidin. It is further alleged that detection of such a hypothetical probe *via* the noncovalent avidin-biotin interaction involves a chemical coupling between the fluorescent molecule (attached to avidin) and the nick-translated, biotinylated probe.

First of all, Applicants disagree with the interpretation of the teaching of the Langer reference as set forth in the rejection. Contrary to the statement on page 6, line 2 of the Office Action, Langer does not teach the use of fluorescently-labeled avidin for the detection of DNA. Rather, Langer discloses that the biotin-avidin interaction had previously been used for detection of proteins, lipids and carbohydrates. Langer discloses biotin-labeled nucleic acids that were detected either by avidin affinity chromatography or by immunoprecipitation with anti-biotin

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antibodies. However, there is no teaching, nor is there any suggestion in Langer relating to the coupling of a fluorescent dye to either biotin or avidin, for use in the detection of a nucleic acid.

Applicants also reiterate their statement regarding the Leary reference, made in the previous response[1], that it is impossible to produce a tagged primer according to the present invention, based on the teaching of Leary. In the nick-translation labeling process used by Leary, biotinylated nucleotides[2] are introduced, by polymerization, into a double-stranded nucleic acid that has undergone random single-strand nicking, utilizing the nicks as sites for initiation of polymerization. Following polymerization, the labeled double-stranded nucleic acid is denatured, to generate a heterogeneous collection of single-stranded fragments whose lengths and sequences are determined by the sites of nicking and the degree of polymerization. Thus, although the method of Leary might produce a collection of labeled **probes**, it is incapable of generating a labeled **primer**, as recited in the present invention.

In this regard, it is important to point out that the term "a primer" is known to one of skill in the art to refer to a population of nucleic acid molecules sharing a common sequence and capable of serving as a locus for initiation of polymerization at a predetermined site on a template. The Office Action appears to be asserting that heterogeneous populations of nucleic acids[3] are also capable, under certain circumstances, of priming polymerization to generate heterogeneous collections of extension products. If such is the case, these heterogeneous **probe** populations are not what one of skill in the art considers "a primer." For this reason, among others, Applicants maintain their traversal of the continued application of this rejection.

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[1] See Applicants' Response dated June 23, 1998 at pages 15-16.

[2] Note that the use of a biotinylated nucleotide by Leary, regardless of how it is introduced into the probe, in and of itself removes Leary as a reference (see below).

[3] Such as those generated by the method of Leary

Another reason for Applicants' traversal of the continued application of this rejection, with respect to the alleged teaching of the Langer and Leary references, is because the structure and properties of the claimed compositions are different from those of the hypothetical primers of Langer and Leary. The claimed compositions include template-primer duplexes comprising oligonucleotides tagged with a chromophore or fluorophore, and extended oligonucleotides derived therefrom. As previously pointed out[4], the tagged compositions of the invention are **inherently detectable**. That is, no additional chemical reactions are necessary for detection of the tagged compositions of the invention. An oligonucleotide labeled with a chromophore is detectable simply by virtue of its color (*i.e.*, light absorption), while emission from a fluorophore-labeled oligonucleotide is detectable upon illumination with light having a wavelength appropriate for the particular fluorophore. One exemplary advantage of the inherent detectability of the claimed compositions is that they can be detected while undergoing electrophoretic separation and other types of biochemical manipulations. This property of the claimed compositions allows, for example, rapid DNA sequence analysis in real time. By contrast, a biotin-labeled oligonucleotide, not being inherently detectable, can only be visualized after the performance of additional chemical reactions, such as binding of a labeled biotin ligand. Such binding reactions generally cannot be conducted during the course of analytical techniques such as electrophoresis, thus precluding any possibility of real-time analysis of biotin-labeled oligonucleotides.

In addition, one embodiment of the invention allows the possibility of labeling different populations of oligonucleotides with different chromophores or fluorophores that are distinguishable by their spectral characteristics[5]. By contrast, it is not possible to distinguish one population of biotin-labeled oligonucleotides from another by virtue of their biotin label.

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[4] See Applicants' Response dated June 23, 1998 at page 15, second full paragraph.

[5] Although the invention is by no means limited to this particular embodiment.

Simply put, the claims recite oligonucleotides tagged with a chromophore or a fluorophore. Nucleic acids produced by the methods of Langer and Leary are tagged with biotin, which is neither a chromophore nor a fluorophore.

Finally, there is absolutely no suggestion in any of these four cited references to support their combination. Qu and Hindley describe primers, while Langer and Leary describe what, at best, might be considered to be probes. Moreover, Langer and Leary are silent with respect to oligonucleotide priming; while Qu and Hindley fail to suggest the desirability even of primers tagged with biotin, let alone with a chromophore and/or a fluorophore.

For all of these reasons, Applicants maintain their traversal of the previously-stated and the present rejection, which rely on hypothetical detection of biotin-labeled nucleic acids, which are incapable of serving as primers, through avidin affinity techniques. Nonetheless, solely to advance prosecution of the application, claims 75, 88, 99, 100, 101-103, 106-107, 111 and 133-138 have been amended to recite covalent coupling between an oligonucleotide and a chromophore or fluorophore. Support is found in Example IV of the application (pages 17-20), wherein the chemical reactions described for the coupling of dyes to an oligonucleotide result in the formation of a covalent linkage. By contrast, the hypothetical primers described in the instant rejection are covalently coupled to a ligand, namely biotin, and are not covalently coupled to a chromophore or fluorophore. Accordingly, Applicants urge that this rejection be withdrawn.

With respect to product-by-process claims 98-103, 105-107, and 127-132; *In re Best*, 562 F.2d 1252; 195 USPQ 430 (CCPA 1977) and *Ex parte Gray*, 10 USPQ2d 1922 (BPAI 1989) are cited to support the assertion that Applicants bear the burden of proving that the claimed compositions are different from those taught in the art and of establishing patentable differences. Applicants maintain that these cases are not applicable to the facts of record. *In re Best* states as follows: "Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require

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an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product." *In re Best*, 562 F.2d 1252; 195 USPQ 430, 433 (CCPA 1977). Inasmuch as the decision in *Gray* is based upon that in *Best*, Applicants maintain that *Best* and *Gray* are inapplicable, since the hypothetical biotinylated nucleic acids described in the Office Action are not identical to the claimed compositions, because the claimed compositions are labeled with a chromophore or a fluorophore and the hypothetical primers on which the rejection is based are labeled with biotin, which is neither a chromophore nor a fluorophore.

Notwithstanding, Applicants believe that the arguments and amendments presented above would have met this burden, had it existed. To summarize, the patentable differences between the claimed labeled oligonucleotides, and the hypothetical labeled oligonucleotides described in the Office Action, are, as stated in the previous Response and reiterated above, that the claimed labeled oligonucleotides are: 1) covalently coupled to a chromophore or a fluorophore, 2) inherently detectable, *i.e.*, detectable without the performance of any additional chemical reaction, and 3) capable of serving as sequence-specific primers. The hypothetical labeled oligonucleotides described in the Office Action possess none of these properties.

B. Claims 75-77, 81-83, 88, 98-103, 105-107, 109-111, and 118-138 stand rejected over Qu or, in the alternative, Hindley, each in view of Smith et al., U.S. Patent No. 5,118,800; issued on June 2, 1992. It is alleged that the effective filing date of the '800 patent is December 20, 1983. Qu and Hindley are applied as above; while the '800 patent is characterized as teaching many reasons for the covalent attachment of a chemical such as a fluorescent dye to an oligonucleotide. It is further stated that the '800 patent specifically teaches methods for generating a 5' amino terminated oligonucleotide, and methods for conjugation of a fluorescent dye to this terminus.

The Office Action states that the effective filing date of the '800 patent is December 20, 1983, because its priority document[6], filed on that date, discloses covalent labeling of oligonucleotides with fluorophores. However, Applicants note that the lineage of the '800 patent includes the filing of two continuation-in-part applications (USSN 709,579 filed March 8, 1985 and USSN 878,045 filed June 24, 1986), which introduced new matter into the disclosure of the 565,010 application. Applicants also note that the presently-pending claims are directed to a duplex comprising a labeled oligonucleotide hybridized to a template, a duplex comprising an extended labeled oligonucleotide hybridized to a template, and a single-stranded oligonucleotide comprising an extended labeled oligonucleotide that has been separated from its template.

A careful review of the '800 patent indicates that, although it discloses the labeling of an oligonucleotide with a fluorescent moiety, it is totally silent with respect to the ability of such a labeled oligonucleotide to hybridize to a template, or to be extended once hybridized. Thus, although the Office Action states that it would have been *prima facie* obvious to have substituted a fluorophore-labeled primer according to the '800 patent for a radioactively-labeled primer in the methods of Qu or Hindley, there is no suggestion in any of the references that a fluorophore labeled primer according to the '800 patent could have been used in the methods of Qu or Hindley either to hybridize to a template or to be extended once hybridized, as is claimed in the present application. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 USPQ 81, 91 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). "At *most*, these articles are invitations to try monoclonal antibodies in immunoassays but do not suggest how that end might be accomplished." (Emphasis in original.) Analogously, the disclosures of either Qu or Hindley, combined with that of the '800 patent, could have done nothing more than provide an invitation to experiment, to determine whether a fluorescently-labeled oligonucleotide is capable of: 1) hybridizing to a template and 2) serving as a primer. The Federal Circuit also stated: "The

[6] U.S. Patent Application Serial No. 565,010.

mere existence of prior art disclosing how to measure the affinity of high affinity monoclonal antibodies is insufficient to support a holding of obviousness. Hybritech's claims define a *process* that *employs* monoclonal antibodies, and does not merely claim antibodies of high affinity." *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 USPQ 81, 92 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). (Emphasis in original.) Similarly, the presently-pending claims recite processes used to produce certain products and the products produced thereby, wherein the processes employ labeled oligonucleotides. The claims do not merely recite labeled oligonucleotides.

Thus, the combined disclosures of the references would not have provided one of skill in the art, at the time the priority application was filed in January 1984, with a likelihood of success at using a chromophore- or fluorophore-labeled oligonucleotide to hybridize to a template, or to be extended once hybridized. To suggest otherwise constitutes improper hindsight reconstruction in light of Applicants' disclosure. For all of these reasons, Applicants believe that this rejection is improper and should be withdrawn.

In light of the inapplicability of this rejection, there is no burden on Applicants, under *In re Best* or *Ex parte Gray*, to establish patentable differences between the claimed subject matter and the hypothetical compositions described in this rejection.

C. Claims 75-77, 81-83, 88, 98, 100-101, 103-105, 107, 109-111, 118-132 and 136-138 stand rejected as allegedly obvious over Qu or, in the alternative, Hindley, each in view of Levinson *et al.* Qu and Hindley are applied as above; while Levinson is alleged to disclose fluorescent labeling of DNA.

As stated in the Office Action, partially depurinated DNA is used for acriflavin labeling by the method of Levinson. The Office Action then goes on to recite, in the next paragraph, ". . . acriflavin labeled primers of Levinson . . ." Thus, an unsupported and incorrect assumption, that DNA labeled according to the method of Levinson could serve as a primer, has been made.

Consideration of the disclosure of Levinson indicates that Levinson's labeled DNA could not serve as a primer. First, the sites of depurination, and hence, of labeling, are random. Second, the extent of depurination and hence, the degree of labeling, is limited. After four hours of depurination, the maximum time disclosed by Levinson, only 1.44 acriflavin molecules are incorporated per 100 bases of DNA (4 x 0.36, see page 265, second paragraph, of Levinson). In certain embodiments of the invention, primers as short as 15 bases or shorter are used. In a population of 15-mers labeled by the method of Levinson, the majority of molecules would be unlabeled. Third, even if the method of Levinson could be used to label DNA to a level at which shorter DNA molecules would be detectable, the extent of depurination required to do so would likely reduce both the extent and specificity of hybridization of the labeled DNA to levels that would be unacceptable for the practice of the invention[7]. Fourth, not only are the sites of labeling random, but the substrate for labeling is a collection of randomly-sheared DNA fragments[8]. In this regard, it is appropriate to state, once again, that the term "a primer" is known to one of skill in the art to refer to a population of nucleic acid molecules sharing a common sequence and capable of serving as a locus for initiation of polymerization at a predetermined site on a template. Heterogeneous probe populations, such as those which might be produced by the method of Levinson, are not what one of skill in the art would consider to be "a primer."

Finally, there is absolutely no suggestion in any of these three cited references to support their combination. Qu and Hindley describe primers, while Levinson describes what at best might be considered to be probes. Moreover, Levinson is silent with respect to oligonucleotide

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[7] See Levinson at page 268, third full paragraph, referring to a "precipitous drop" in the reassociation of his labeled DNA after only 3 hours of depurination. See also page 271, fourth full paragraph.

[8] See Levinson at page 261, paragraph entitled "Preparation of DNA."

priming; while Qu and Hindley fail to suggest the desirability of primers tagged with chromophores and/or fluorophores.

In light of the inapplicability of this rejection, there is no burden on Applicants, under *In re Best* or *Ex parte Gray*, to establish patentable differences between the claimed subject matter and the hypothetical compositions described in this rejection.

For all of the aforementioned reasons, Applicants believe that this rejection is improper and urge that it be withdrawn.

CONCLUSION

Applicants have, by way of the amendments and remarks presented herein, made a sincere effort to overcome all outstanding rejections and address all issues that were raised in the outstanding Office Action. Accordingly, reconsideration and allowance of the pending claims are respectfully requested. If it is determined that a telephone conversation would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the unlikely event that the transmittal letter is separated from this document and/or the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief, including extensions of time, and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 243132000105. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: February 26, 1999 By: Sean Brennan

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APPENDIX A

U.S.S.N. 08/484,340

Pending claims

February, 1999

75. (Thrice amended) A duplex comprising an oligonucleotide primer and a template, wherein the primer is covalently coupled to a chromophore or fluorophore so as to allow chain extension by a polymerase.

76. (Thrice Amended) A duplex comprising a template and an extended primer, produced by providing a duplex according to claim 75 and extending the primer with a polymerase.

77. (Thrice amended) A single-stranded oligonucleotide produced by separating the extended primer of claim 76 from the template.

81. (Twice Amended) A set of duplexes comprising two or more of the duplexes of claim 75.

82. (Twice Amended) A set of duplexes comprising two or more of the duplexes of claim 76.

83. (Twice Amended) A set of oligonucleotides comprising two or more of the oligonucleotides of claim 77.

88. (Thrice Amended) A set of reagents comprising oligonucleotide primers covalently coupled to one or more chromophores or fluorophores so as to allow chain extension by a polymerase, and a polymerase.

98. (Twice Amended) A single-stranded oligonucleotide comprising a first portion and a second portion,

wherein the first portion comprises an oligonucleotide fragment covalently coupled to a chromophore or fluorophore; and

wherein the second portion is produced by extension of the first portion along a complementary template.

99. (Twice amended) The oligonucleotide of claim 98, wherein the chromophore or fluorophore is covalently coupled to the first portion through an amine linkage.

100. (Twice amended) The oligonucleotide of claim 98, wherein the chromophore or fluorophore is covalently coupled to the first portion at its 5' end.

101. (Thrice Amended) The duplex of claim 75, prepared by a method comprising:

hybridizing a primer to a template, wherein the primer is covalently coupled to a chromophore or fluorophore so as to allow chain extension by a polymerase.

102. (Thrice Amended) The duplex of claim 101, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

103. (Thrice amended) The duplex of claim 101, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

105. (Thrice amended) An oligonucleotide produced by the method comprising extending the primer of claim 75 by a polymerase to produce an oligonucleotide and separating the oligonucleotide from the template.

106. (Four times amended) The oligonucleotide of claim 105, wherein the chromophore or fluorophore is covalently coupled to the oligonucleotide through an amine linkage.

107. (Four times amended) The oligonucleotide of claim 105, wherein the chromophore or fluorophore is covalently coupled to the oligonucleotide at its 5' end.

109. (Four Times Amended) A chain termination method comprising extending the primer of claim 75 by a polymerase to produce an extended primer and separating the extended oligonucleotide from the template.

110. (Thrice Amended) A chain termination method comprising extending the set of primers of claim 81 by a polymerase to produce a set of extended primers.

111. (Four times amended) The chain termination method of claim 110, wherein the reaction comprises four chain termination DNA sequencing reactions, and the covalently coupled oligonucleotides comprising each of the four reactions are distinguishable by spectral characteristics of the chromophore or fluorophore.

118. (Twice amended) The oligonucleotide of claim 77, wherein the primer is DNA.

119. (Twice amended) The oligonucleotide of claim 77 wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

120. (Twice amended) The duplex of either of claims 75 or 76, wherein the chromophore or fluorophore is detectable by exposure to a laser.

121. (Twice amended) The set of duplexes of either of claims 81 or 82, wherein the primers are DNA.

122. (Twice amended) The set of duplexes of either of claims 81 or 82, wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

123. (Twice amended) The set of duplexes of either of claims 81 or 82, wherein the chromophore or fluorophore is detectable by exposure to a laser.

124. (Amended) The set of reagents of claim 88, wherein the primers are DNA.

125. (Amended) The set of reagents of claim 88, wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

126. (Amended) The set of reagents of claim 88, wherein the chromophore or fluorophore is detectable by exposure to a laser.

127. (Amended) The oligonucleotide of any of claims 105 to 107, wherein the primer is DNA.

128. (Amended) The oligonucleotide of any of claims 105 to 107 wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

129. (Amended) The oligonucleotide of any of claims 105 to 107, wherein the chromophore or fluorophore is detectable by exposure to a laser.

130. (Amended) The duplex of any of claims 101 to 103, wherein the primer is DNA.

131. (Amended) The duplex of any of claims 101 to 103, wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

132. (Amended) The duplex of any of claims 101 to 103, wherein the chromophore or fluorophore is detectable by exposure to a laser.

133. (Amended) The duplex of either of claims 75 or 76, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

134. (Amended) The set of duplexes of either of claims 81 or 82, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

135. (Amended) The set of reagents of claim 88, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

136. (Amended) The duplex of either of claims 75 or 76, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

137. (Amended) The set of duplexes of either of claims 81 or 82, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

138. (Amended) The set of reagents of claim 88, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

139. (New) The oligonucleotide of claim 77, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

140. (New) The oligonucleotide of claim 77, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

141. (New) The oligonucleotide of claim 77, wherein the chromophore or fluorophore is detectable by exposure to a laser.

142. (New) The set of oligonucleotides of claim 83, wherein the primers are DNA.

143. (New) The set of oligonucleotides of claim 83, wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

144. (New) The set of oligonucleotides of claim 83, wherein the chromophore or fluorophore is detectable by exposure to a laser.

145. (New) The set of oligonucleotides of claim 83, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

146. (New) The set of oligonucleotides of claim 83, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

APPENDIX A

U.S.S.N. 08/484,340

Pending claims

February, 1999

75. (Thrice amended) A duplex comprising an oligonucleotide primer and a template, wherein the primer is covalently coupled to a chromophore or fluorophore so as to allow chain extension by a polymerase.

76. (Thrice Amended) A duplex comprising a template and an extended primer, produced by providing a duplex according to claim 75 and extending the primer with a polymerase.

77. (Thrice amended) A single-stranded oligonucleotide produced by separating the extended primer of claim 76 from the template.

81. (Twice Amended) A set of duplexes comprising two or more of the duplexes of claim 75.

82. (Twice Amended) A set of duplexes comprising two or more of the duplexes of claim 76.

83. (Twice Amended) A set of oligonucleotides comprising two or more of the oligonucleotides of claim 77.

88. (Thrice Amended) A set of reagents comprising oligonucleotide primers covalently coupled to one or more chromophores or fluorophores so as to allow chain extension by a polymerase, and a polymerase.

98. (Twice Amended) A single-stranded oligonucleotide comprising a first portion and a second portion,

wherein the first portion comprises an oligonucleotide fragment covalently coupled to a chromophore or fluorophore; and

wherein the second portion is produced by extension of the first portion along a complementary template.

99. (Twice amended) The oligonucleotide of claim 98, wherein the chromophore or fluorophore is covalently coupled to the first portion through an amine linkage.

100. (Twice amended) The oligonucleotide of claim 98, wherein the chromophore or fluorophore is covalently coupled to the first portion at its 5' end.

101. (Thrice Amended) The duplex of claim 75, prepared by a method comprising:

hybridizing a primer to a template, wherein the primer is covalently coupled to a chromophore or fluorophore so as to allow chain extension by a polymerase.

102. (Thrice Amended) The duplex of claim 101, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

103. (Thrice amended) The duplex of claim 101, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

105. (Thrice amended) An oligonucleotide produced by the method comprising extending the primer of claim 75 by a polymerase to produce an oligonucleotide and separating the oligonucleotide from the template.

106. (Four times amended) The oligonucleotide of claim 105, wherein the chromophore or fluorophore is covalently coupled to the oligonucleotide through an amine linkage.

107. (Four times amended) The oligonucleotide of claim 105, wherein the chromophore or fluorophore is covalently coupled to the oligonucleotide at its 5' end.

109. (Four Times Amended) A chain termination method comprising extending the primer of claim 75 by a polymerase to produce an extended primer and separating the extended oligonucleotide from the template.

110. (Thrice Amended) A chain termination method comprising extending the set of primers of claim 81 by a polymerase to produce a set of extended primers.

111. (Four times amended) The chain termination method of claim 110, wherein the reaction comprises four chain termination DNA sequencing reactions, and the covalently coupled oligonucleotides comprising each of the four reactions are distinguishable by spectral characteristics of the chromophore or fluorophore.

118. (Twice amended) The oligonucleotide of claim 77, wherein the primer is DNA.

119. (Twice amended) The oligonucleotide of claim 77 wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

120. (Twice amended) The duplex of either of claims 75 or 76, wherein the chromophore or fluorophore is detectable by exposure to a laser.

121. (Twice amended) The set of duplexes of either of claims 81 or 82, wherein the primers are DNA.

122. (Twice amended) The set of duplexes of either of claims 81 or 82, wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

123. (Twice amended) The set of duplexes of either of claims 81 or 82, wherein the chromophore or fluorophore is detectable by exposure to a laser.

124. (Amended) The set of reagents of claim 88, wherein the primers are DNA.

125. (Amended) The set of reagents of claim 88, wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

126. (Amended) The set of reagents of claim 88, wherein the chromophore or fluorophore is detectable by exposure to a laser.

127. (Amended) The oligonucleotide of any of claims 105 to 107, wherein the primer is DNA.

128. (Amended) The oligonucleotide of any of claims 105 to 107 wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

129. (Amended) The oligonucleotide of any of claims 105 to 107, wherein the chromophore or fluorophore is detectable by exposure to a laser.

130. (Amended) The duplex of any of claims 101 to 103, wherein the primer is DNA.

131. (Amended) The duplex of any of claims 101 to 103, wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

132. (Amended) The duplex of any of claims 101 to 103, wherein the chromophore or fluorophore is detectable by exposure to a laser.

133. (Amended) The duplex of either of claims 75 or 76, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

134. (Amended) The set of duplexes of either of claims 81 or 82, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

135. (Amended) The set of reagents of claim 88, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

136. (Amended) The duplex of either of claims 75 or 76, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

137. (Amended) The set of duplexes of either of claims 81 or 82, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

138. (Amended) The set of reagents of claim 88, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

139. (New) The oligonucleotide of claim 77, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

140. (New) The oligonucleotide of claim 77, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

141. (New) The oligonucleotide of claim 77, wherein the chromophore or fluorophore is detectable by exposure to a laser.

142. (New) The set of oligonucleotides of claim 83, wherein the primers are DNA.

143. (New) The set of oligonucleotides of claim 83, wherein the chromophore or fluorophore is detectable by exposure to a high-intensity monochromatic light source.

144. (New) The set of oligonucleotides of claim 83, wherein the chromophore or fluorophore is detectable by exposure to a laser.

145. (New) The set of oligonucleotides of claim 83, wherein the chromophore or fluorophore is covalently coupled to the primer through an amine linkage.

146. (New) The set of oligonucleotides of claim 83, wherein the chromophore or fluorophore is covalently coupled to the primer at its 5' end.

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